



*This is a post-peer-review, pre-copyedit version of an article published in Trends in Plant Science. The final authenticated version is available online at: <https://doi.org/10.1016/j.tplants.2020.04.004>*

# Emerging Connections between Small RNAs and Phytohormones

**Ting Li,<sup>1,2</sup> Nathalie Gonzalez,<sup>3</sup> Dirk Inzé,<sup>1,2,\*</sup> and Marieke Dubois,<sup>1,2</sup>**

<sup>1</sup> Ghent University, Department of Plant Biotechnology and Bioinformatics, 9052 Ghent, Belgium

<sup>2</sup> VIB Center for Plant Systems Biology, 9052 Ghent, Belgium

<sup>3</sup> INRAE, Univ. Bordeaux, BFP, F33882 Villenave d'Ornon, France

\* Correspondence: [dirk.inze@psb.vib-ugent.be](mailto:dirk.inze@psb.vib-ugent.be) (D. Inzé), Website: <https://www.psb.ugent.be/systems-biology-of-yield>, Twitter: @InzeDirk

**Keywords:** miRNA, siRNA, sRNA, hormones, development, stress response

**Small RNAs (sRNAs), mainly including microRNAs (miRNAs) and small interfering RNAs (siRNAs), are ubiquitous in eukaryotes. sRNAs mostly negatively regulate gene expression via (post-)transcriptional gene silencing through DNA methylation, mRNA cleavage, or translation inhibition. The mechanisms of sRNA biogenesis and function in diverse biological processes, as well as the interactions between sRNAs and environmental factors, like (a)biotic stress, have been deeply explored. Phytohormones are central in the plant's response to stress, and multiple recent studies highlight an emerging role for sRNAs in the direct response to or the regulation of plant hormonal pathways. In this review, we discuss recent progress on the unraveling of cross-regulation between sRNAs and nine plant hormones.**

## Highlights

- An increasing number of studies identified a large variety of sRNAs responding to diverse phytohormones, and in-depth validation revealed molecular mechanisms underneath this.
- Conversely, multiple sRNAs and central proteins in sRNA pathways can regulate biosynthesis or signaling of nine phytohormones.
- Some sRNA modules interconnect with more than one hormonal pathway, thereby providing new bridges in plant hormonal cross-talk.
- In response to environmental stimuli, phytohormones enable plant adaptation and part of this reaction could be attributed to sRNAs and their targets

### **Small RNAs and Hormones: two Mutually Influenced Systems for Plant Growth and Stress Response**

Phytohormones are important signaling molecules involved in almost all biological processes of the plant's life cycle [1-5]. To date, auxin, ethylene (Et), gibberellic acid (GA), cytokinin (Ck), abscisic acid (ABA), brassinosteroids (BR), jasmonic acid (JA), salicylic acid (SA) and strigolactones (SL) have been identified as the main plant hormones. They are synthesized via different routes and are perceived by receptor proteins, which subsequently initiate intracellular signal transduction [6]. Ultimately, the transduction reaches transcription factors (TFs) that control the downstream hormonal response. Hormones cooperate to modulate diverse processes including vascular root patterning, cell elongation, abiotic stress response, or biotic stress defense [7-12].

Other endogenous molecules also participate to all these biological processes: the sRNAs, 18-25nt in length RNAs mainly consisting of siRNAs and miRNAs (Box 1) [13]. sRNAs constitute important regulators of plant development under favorable conditions, for example, for the establishment of leaf patterning and leaf growth [14-17]. Moreover, they also participate to environmental stress responses. For example, upon virus infection, siRNAs trigger the cleavage of viral RNAs to protect the plant, while miRNAs participate by silencing the negative regulators of the plant's immune system [18,19]. sRNAs are pivotal in abiotic stress response as well. Numerous sRNAs are produced upon abiotic stress exposure and in turn regulate the expression of genes involved in stress defense [20,21].

Even though the metabolic and transduction pathways of hormones and sRNAs are very different, they participate in common biological processes and multiple recent studies highlighted interplay between hormones and sRNAs. Their connections enable plants to rapidly and efficiently adapt to environmental stresses by opting for sRNAs as intermediates to control hormone levels or, conversely, by using hormones to modulate the levels of specific sRNAs. Aiming at overviewing these new connections, we discuss the involvement of sRNAs in the regulation of biosynthesis and signaling of the nine major phytohormones, grouped based on their biological function (Table 1).

### **sRNAs Form Novel Hubs in Growth-Promoting Hormonal Networks**

#### *Gibberellins*

GAs are crucial for developmental processes like seed germination, stem elongation and flower initiation. They are synthesized through the activity of GA-OXIDASES (GA20OX and GA3OX) and perceived by the receptor that promotes degradation of DELLA proteins, key repressors of the GA response [22]. Treating plants with GA not only affects the level of protein-coding transcripts, but also triggers the production of more than hundred miRNAs in plants [23,24] (Table 1). How this GA-mediated control of sRNA levels occurs, as well as whether sRNAs can act on GA biosynthesis and signaling, has attracted researchers' attention.

Because they are central factors in GA response, DELLAs were proposed to mediate the regulation of several sRNAs via interaction with protein partners. A first example is the os-miR396 of rice (*Oryza sativa*) that is directly induced by INDETERMINATE DOMAIN2 (IDD2). IDD2 interacts directly with the rice DELLA protein SLENDER RICE1, and the induction of os-miR396 is disabled upon GA treatment, when DELLAs are absent, strongly suggesting that DELLAs are necessary for the regulation of this sRNA (Figure 1). The induction of os-miR396 further reduces the expression of its targets, the *GROWTH-REGULATING FACTORS* (*GRFs*) transcription factors involved in growth promotion. Consistently, os-miR396 overexpression results in dwarfism that resembles GA-deficient plants [25]. Secondly, DELLAs can also function through the degradation of PIF4 that regulates miRNA levels via binding to the *MIR* promoter or via destabilizing the miRNA-processing complex [26,27]. As such, the *MIR172a* overaccumulates in the *pif4* arabidopsis mutant (*Arabidopsis thaliana*), thus, PIF4 is a negative regulator of ath-miR172 [27] (Figure 1). In contrast, its homolog *MIR172b* is

repressed by the DELLA protein, pointing towards a regulatory mechanism different from *MIR172a*. This inhibition of *MIR172b*, which causes flowering delay, might be indirect via the regulation of the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) (Figure 1). DELLAs directly bind and thereby inhibit the activity of SPLs, transcription factors that positively regulate ath-miR172 and that are, in turn, targeted by ath-miR156 [28]. Finally, a third possible DELLA-regulated sRNA is ath-miR171, a sRNA targeting the transcription factor SCARECROW-LIKE27 (SCL27), which elevates ath-miR171 levels via a negative feedback loop [29] (Figure 1). ath-miR171 abundance is decreased in *pif4* mutants [27], and an independent study shows that DELLA interferes with the activity of SCL27 in chlorophyll biosynthesis. Thus, by regulating SCL27 activity, DELLA might negatively affect the ath-miR171 [29].

In turn, GA-biosynthesis genes can be positively affected by sRNAs indirectly, and sl-miR171 is one of them. Similarly to the module in arabidopsis, an SCL6-ortholog in tomato (*Solanum lycopersicum*), *SIGRAS24*, is targeted by sl-miR171 (Figure 1). *SIGRAS24* expression is quickly elevated by GA, leading to a reduction in the expression of *SIGA20OX1* and *SIGA3OX1*, which results in a GA-deficiency phenotype [30]. In contrast, one sRNA is reported to negatively regulate GA synthesis. The wheat (*Triticum*)-specific tri-miR9678 triggers a delay in seed germination by decreasing the expression of GA biosynthesis genes [31].

Finally, GA signaling can be regulated by a negative feedback loop involving one sRNA in particular: ath-miR159, whose expression is induced by GA in arabidopsis [32]. In turn, ath-miR159 targets *GAMYB* or *GAMYB-like* transcription factors involved in GA signaling, affecting also their downstream targets. For example, *LEAFY*, a potential target of MYB33 that is induced by GA, is negatively regulated by ath-miR159 (Figure 1). *LEAFY* stimulates transition to flowering and, consequently, overexpression of ath-miR159 in the Landsberg arabidopsis accession delays flowering in short-day conditions [33]. In rice, os-miR159 can also affect GA biosynthesis. Although os-miR159 level is not altered by GA treatment, os-miR159 has a positive effect on GA biosynthesis by cleaving its target *OsGAMYBL2*, a negative regulator of GA biosynthesis [34]. Taken together, the miR159-GAMYB(L)s module seems to constitute a key modulator of GA response, also affecting GA biosynthesis in some species.

In conclusion, the phytohormone GA can alter the level of multiple sRNAs. This might occur in part via DELLA proteins and their interactors, such as IDD2, PIF4 or SCL. To explore the extent of DELLA-mediated sRNA regulation, a comparison of the miRNA content of DELLA gain-of-function plants with that of *della* mutants could be helpful. In turn, sRNAs are able to directly regulate GA biosynthesis and signaling via miR156-SPL, miR171-SCL and miR159-GAMYB(L)s modules, respectively.

### *Brassinosteroids*

BRs are steroid hormones mainly involved in plant growth, vascular differentiation and stomatal development [35]. In arabidopsis, miRNAs from 48 known families and 23 unknown miRNAs are differentially expressed upon BR treatment [36] (Table 1). While the role of these BR-induced sRNAs has not been studied in arabidopsis yet, recent research performed in rice has shown that, conversely, sRNAs influence BR synthesis and signaling.

sRNAs can directly target transcripts of BR biosynthesis and signaling genes for cleavage. As such, OsDCL3a (Box 1) produces 24-nt siRNAs from transposable elements, resulting in the downregulation of the BR-biosynthesis gene *OsBR6ox*, and reduced BR levels [37] (Figure 1). Moreover, the os-miR1848 silences *OsCYP51G3* transcripts, encoding a cytochrome P enzyme that mediates BR biosynthesis. Consequently, overexpression of this miRNA causes BR deficiency under salt stress condition [38]. Also in rice, a line overexpressing os-miR397 shows higher grain yield and hypersensitivity to BR. This might be attributed to the cleavage of the target gene *OsLAC*, encoding a laccase-like protein involved in BR-related gene expression [39]. In contrast, os-miR444 induces BR-biosynthetic genes by silencing their transcriptional repressor *OsMADS57* and, thereby, promotes BR-mediated inhibition of root elongation [40] (Figure 1).

Remarkably, two sRNAs bridge BR with GA, contributing to the control of rice architecture and grain yield. Upon BR treatment, the level of os-miR159 rapidly decreases, leading to accumulation of *OsGAMYBL2*, which stabilizes the ortholog of the BRASSINOSTEROID-INSENSITIVE2 kinase. Interestingly, this also leads to decreased expression of the positive BR response regulator *BRASSINOSTEROID UPREGULATED1* and to inhibition of GA biosynthesis [34] (Figure 1). Moreover, GA biosynthesis can also be inhibited by BR via another sRNA-mediated pathway. The

BR-responsive TF BRASSINAZOLE-RESISTANT (OsBZR1) directly promotes the expression of *OsMIR396d*, which results in silencing of *OsGRF6* and reduced expression of GA-biosynthesis genes *OsGA20OX* and *OsGA3OX* [41] (Figure 1).

Altogether, evidence obtained from studies in rice suggest that BR biosynthesis and signaling can be controlled via siRNA- and miRNA-mediated mechanisms. However, the link between sRNAs and BR, as well as the involvement of sRNAs in GA-BR crosstalk in other species still need further investigation and genetic validation to understand the involvement of the different modules in these interactions.

### *Auxin*

Auxin (INDOLE-3-ACETIC ACID, IAA) is another pivotal hormone that contributes mainly to root patterning and leaf morphology. It can be synthesized via flavin-containing monooxygenases (YUCCA, YUC) and transported via auxin influx and efflux carriers. Auxin signaling is then initiated by the co-receptors TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB), which interacts with the Aux/IAA transcriptional repressors to promote their degradation, releasing AUXIN RESPONSE FACTORS (ARFs) [42]. Interestingly, the miRNA biogenesis mutant *hyl1* (Box 1) exhibits reduced sensitivity to exogenous auxin and the *ago1* mutant shows less IAA accumulation in roots [43,44]. These findings indicate that sRNAs and regulation of auxin are tightly linked and raise the questions of whether sRNAs affect auxin biosynthesis or transduction, which sRNAs are involved, and how plants integrate these two signals to precisely orchestrate plant morphology (Figure 1).

While the lower IAA level in *ago1* mutants suggest that sRNAs are positively involved in auxin biosynthesis [43], few sRNAs are robust candidates for this regulation. One notable exception is *ath-miR10515*, which stimulates IAA production by downregulating its target gene *SUPERROOT1* (*SUR1*) that encodes an enzyme antagonizing IAA production (Figure 1). Consequently, IAA-responsive genes are highly expressed in the *ath-miR10515* overexpression line and reduced in *ath-miR10515* mutant [45]. On the contrary, several miRNAs decrease auxin levels. As such, *miR165/166* silences *REVOLUTA* (*REV*) transcripts, which encode a direct positive regulator of *YUC5* [46] (Figure 1). Similarly, in rice, attenuating *OsMIR396d* or *OsMIR1432* levels causes an increase of auxin-biosynthesis gene expression or of

auxin transport, resulting in plants performing better in terms of grain filling and yield [41,47]. Finally, *IAA-Ala RESISTANT3 (IAR3)*, encoding an enzyme that releases bioactive auxin, is targeted by the ath-miR167, a miRNA that mainly targets ARFs [48] (Figure 1). As *IAR3* is induced by high osmotic stress and contributes to drought tolerance, whereas ath-miR167 is downregulated under stress, this module could participate in fine-tuning auxin levels under water-limiting conditions. Besides these miRNAs, also siRNAs can negatively affect auxin biosynthesis. *YUC2* expression is controlled by thermo-regulated heterochromatic siRNAs (hc-siRNAs) (Figure 1). With increasing temperature, the level of hc-siRNAs able to bind to the *YUC2* promoter is reduced, leading to the elevation of *YUC2* expression. This hc-siRNAs–*YUC2* pattern is consistent with previous observations showing that auxin accumulates upon higher temperature [49].

The reduced root elongation in *hyl1* in response to IAA treatment indicates that sRNAs could also be involved in auxin response and, indeed, several sRNAs directly target almost all auxin signaling members, from the receptor to the transcriptional regulators. For example, ath-miR393 and secondary ath-siRNAs promote *TIR1/AFB* transcript degradation [50], while the Aux/IAA IAA28 transcripts are recognized by ath-miR847 [51] (Figure 1). However, these miRNAs are not affected in the *hyl1* mutant, so they cannot explain *hyl1*'s auxin-hyposensitive phenotype. More downstream, multiple ARFs are targeted by sRNAs forming a crucial network in plant development [52-55]. A well-documented example is the ath-miR165/166-ARF3/4 module in the establishment of adaxial-abaxial polarity of leaves. The ath-miR165/166 targets *PHABULOSA (PHB)*, a direct activator of ARF5, which in turn triggers the expression of *MIR390* [56,57] (Figure 1). Increased miR390 levels directly cause accumulation of tasi-RNAs of ARF3/4 (tasi-ARF3/4) [58]. The opposite movement of both sRNAs, ath-miR165/166 and ath-tasiARF3/4, along the adaxial/abaxial axis of the leaf, alters the distribution of ARF3/4 and PHB/REV, creating robust developmental boundaries for maintaining a flat leaf architecture [59].

Overall, these examples demonstrate a large involvement of sRNAs at multiple levels of auxin biosynthesis and signaling (Table 1), explaining why compromising sRNA biogenesis can alter auxin sensitivity. However, the available literature currently does not explain why plants lacking miRNAs (*hyl1*) are auxin-hyposensitive, as the overall effects of sRNAs on the auxin pathways are negative. Another exciting question is how

sRNA spatial dynamics might help to establish the specific auxin distribution, a point that has greatly contributed to our understanding of leaf polarity, but that has not been explored in roots yet. Finally, with the exception of the *ARF5-miR390* module, our knowledge on if and how auxin or ARFs control the expression of sRNAs is still rather limited.

### *Cytokinin*

CKs were discovered because of their pivotal role in cell division. CKs are synthesized via the adenylate-isopentenyltransferase (IPT), activated by the enzyme LONELY GUY (LOG), and perceived by receptors. This results in the activation of the transcriptional regulators, B-Type ARABIDOPSIS RESPONSE REGULATORS (ARRs), thereby triggering the induction of CK-responsive genes [60]. In addition to the reduced sensitivity to auxin treatment, *hy1* exhibits hyposensitivity to CK, raising the question of how sRNAs contribute to the control of CK response [44].

sRNAs seem to affect CK biosynthesis and, to a lesser extent, CK signaling. For example, *st-miR156* increases CK levels by indirectly inducing *LOG1* in potato (*Solanum tuberosum*), which results in more pronounced CK-induced branching [61] (Figure 1). Oppositely, *ath-miR159* and *ath-miR319* suppress the expression of *SHOOTMERISTEMLESS* and *BREVIPEDICELLUS*, which enhances *IPT* expression and promotes CK biosynthesis in the shoot apical meristem [62,63]. Additionally, in tomato, the *sl-miR208* directly silences *IPT2*, causing a reduction in CK level and an early leaf senescence phenotype [64]. Besides miRNAs, also siRNAs contribute to the regulation of CK biosynthesis. In petunia (*Petunia hybrida*), anti-sense transcription of the *Sho (PhIPT)* locus generates natural cis-antisense siRNAs (nat-siRNAs). They target *Sho* sense transcripts, encoding an enzyme responsible for CK biosynthesis [65] (Figure 1). At CK transduction level, the *ath-miR156*-target *SPL9* inhibits B-type ARRs, hence, the *ath-miR156-SPL9* module regulates CK-related shoot regenerative capacity [66] (Figure 1).

Notably, sRNAs also affect CK biosynthesis and transduction via auxin. This is particularly interesting as sRNA biogenesis mutants are hyposensitive to both hormones, suggesting that sRNAs are involved in maintaining the CK/auxin balance. For example, the above-described *ath-miR165* target, *PHB*, not only promotes auxin response but also CK accumulation via activation of *IPT7*. In turn, *ARR1* prevents *PHB*



and *MIR165a* expression for the inhibition of root growth [67] (Figure 1). Another example is the *ath-miR160*-target *ARF10* that promotes callus formation through direct repression of a negative regulator of CK response [68]. Supporting this, in soybean (*Glycine max*), *gm-miR160*-overexpression inhibits CK-related nodule development [69]. These examples suggest that miRNAs participate in the CK/auxin crosstalk.

Conversely, very few studies explored how CK affects sRNAs. In the context of nodule initiation, CKs trigger the induction of *NODULATION SIGNALING PATHWAY2* (*NSP2*) genes, that are responsible for nodule formation. In parallel, however, CKs also stimulate the medicago (*Medicago truncatula*) *miR171h*, which is capable of *NSP2* silencing. Therefore, this *miR171h*-*NSP2* module forms a balance mechanism in the control of nodule initiation [70,71].

In conclusion, several studies pointed out a direct effect of sRNAs on CK at the level of CK biosynthesis in multiple species (Table 1). In most known cases, this occurs through sRNA-mediated control of *IPT* genes, encoding rate-limiting enzymes in CK biosynthesis. sRNAs also have a role in modulating the auxin/CK balance in different tissues, with the *ath-miR165* emerging as an additional player in the auxin/CK homeostasis in root growth regulation. Whether other sRNAs affect this balance in other tissues or organs forms an exciting question for future research. Answering this might contribute to a better understanding of why sRNA mutants have altered sensitivity to both hormones.

### *Strigolactones*

Unlike other phytohormones, strigolactones (SLs), involved in shoot branching, were discovered relatively recently. In rice, SLs are perceived by the  $\alpha/\beta$ -hydrolase *DWARF14* (*D14*), which stimulates the degradation of *D53*, causing the induction of SL-responsive genes [72]. Although not many connections between SLs and sRNAs have been elucidated yet, the expression of key proteins in SL signaling is partly controlled by sRNAs (Table 1).

In rice, the SL suppressor *D53* interacts with the *os-miR156*-target *SPL14* and suppresses its transcriptional activity, while *SPL14* positively regulates *D53* (Figure 1). Consequently, *os-miR156*-overexpressing plants have more branches and are SL insensitive [73,74]. Furthermore, the arabidopsis *D53*-orthologs, *SMXL4/5*, act

as templates for RDR6-DCL2-dependent siRNAs biogenesis (Box 1). Interestingly, DCL4, the homolog of DCL2, plays a negative role in the generation of these *SMXL4/5*-derived ath-siRNAs (Figure 1). Hence, these siRNAs accumulate in the *dc14* mutant, leading to the silencing of *SMXL4/5* and the phenocopy of the *smx14smx15* double mutant [75].

Although our current knowledge about how sRNAs and strigolactones interplay is still very limited, the miR156-SPL module sheds light on a new level of post-transcriptional regulation of SLs. When further uncovering the SL pathways, it will be interesting to consider the potential regulatory role of these miRNAs and siRNAs, as they can affect key transcripts, such as those of *SMXL4/5*.

### **sRNAs Coordinate Salicylic and Jasmonic Acid in Response to Biotic Stress**

When plants are infected by pathogens, the PATTERN RECOGNITION RECEPTORS detect damage-associated molecular patterns and stimulate plant immunity [76]. In this context, SA, JA, and sRNAs are induced to counteract the pathogen invasion [8,9,19]. Interestingly, *hyl1* mutants show over-activated JA signaling, and *HYL1*-overexpressing plants are more susceptible to *Botrytis cinerea* infection [77]. Moreover, knocking-out RNA Pol V subunits (Box 1) leads to reduced induction of JA-responsive genes and more pronounced induction of SA-responsive genes upon infection with the bacterial pathogen *Plectosphaella cucumerina* [78,79]. These observations raise the question of whether JA, SA and sRNAs are connected in the defense response towards pathogens, besides their well-known role in silencing of viral RNA (Box 2).

Upon pathogen infection, SA, a phenolic compound crucial for plant pathogen defense, accumulates and is perceived by the receptor NONEXPRESSER OF PR-GENES (NPR), activating transcription factors called TGACGTCA CIS-ELEMENT-BINDING PROTEIN (TGA) and WRKY DNA-BINDING PROTEIN (WRKY) [80]. Interestingly, the promoters of *AGO*, *DCL* and *RDR* genes contain predicted binding sites for TGAs and WRKYs. Accordingly, SA application triggers changes in *AGO1* and *DCL2/3/4* transcript levels, suggesting SA can affect sRNAs in a wider context than upon virus invasion, although the biological consequences of these expression changes are unclear [81]. Conversely, increasing evidence suggests that SA levels and signaling can be affected by endogenous sRNAs and that pathogens can misuse this system. In tomato, sl-miR396a, which targets *GRF1* transcripts to reduce *TGA1/2* and

*PATHOGEN-RELATED1 (PR)* transcript levels, is repressed upon fungal infection (Figure 2). Consequently, sl-miR396-overexpressing tomato plants are more susceptible to *Phytophthora infestans* and *Botrytis cinerea* infection, even though they show a higher SA concentration and *NPR* expression [82].

Also related to biotic stress, JA is responsible for the wounding response and antagonizing insect attacks. JA biosynthesis occurs by the conversion of unsaturated fatty acids into JA via diverse enzymes, such as lipoxygenase (LOX). Triggered by JA, the CORONATINE INSENSITIVE1-JASMONATE-ZIM-DOMAIN PROTEIN (COI1-JAZ) co-receptor complex is activated and elicits degradation of the suppressor JAZ, allowing transcription factors (i.e. MYC2) to promote JA-responsive genes [83]. Main regulators of sRNAs function, like AGO1 and HSP70/90, are emerging as positive regulators of the JA signaling pathway; AGO1 incorporates sRNAs derived from JA-responsive genes, like JAZ, MYC2 or LOX2 and promotes these JA-responsive genes' expression in response to JA application [84] (Figure 2). Moreover, HSP70/90 can stabilize COI1 to stimulate the JA response [85]. In turn, two JA-induced sRNAs, miR319 and ath-miR156, provide feedback regulation in the JA pathway. First, miR319 targets *TCP4* in arabidopsis and tomato, and *TCP21* in rice, thereby inhibiting *LOX* in response to biotic stress, which alters the sensitivity to diverse pathogens [86-88]. More downstream, ath-miR156 targets the gene encoding SPL9, which physically interacts with JAZ3 and promotes JAZ3 stability, resulting in attenuated insect resistance [89] (Figure 2).

Because these two hormones participate in the plant's immunity, SA and JA are influenced by RNA silencing suppressor (RSS) encoded by viruses for antagonizing virus-activated siRNA pathways (Box 2). For example, *Cauliflower mosaic virus (CaMV)* P6 protein suppresses SA accumulation by activation of TARGET OF RAPAMYCIN, which downregulates the expression of *NPR1* and *WRKY45* [90,91] (Figure 2). An alternative strategy is used by the *Turnip mosaic virus*, where RSS Hc-Pro physically interacts with a homolog of SA-BINDING PROTEIN and represses the SA-mediated immune response [92]. On the contrary, *Potato virus A* Hc-Pro and *Geminiviridae* AC2, another RSS, promote expression of JA biosynthesis- and JA-related genes, like *LOX* and *VSP1* [93,94], while several other RSSs were reported to hinder the JA response [95,96].

In conclusion, it is clear that several recent evidences suggest a role for sRNAs, both from endogenous and viral origin, in the fine-tuning of JA and SA levels and response during biotic stress defense (Table 1). However, except for Pol V subunits, no other endogenous sRNA regulators are known to affect both JA as well as SA, but this would be an interesting question for future studies. On the other hand, only few studies reported the effect of SA/JA on sRNA levels, which raises the question of whether these hormones mainly act downstream of sRNAs. This is most likely not the case, as recent sRNAseq data in arabidopsis revealed that 87 ath-miRNAs and 4 ath-tasiRNAs show differential expression upon JA treatment [97]. Further characterizing the functional importance of these sRNAs in plant immunity is an exciting area for future research, both from an academic and a more applied point of view, as engineering these sRNAs could contribute to increased resistance to plant pathogens.

### **sRNAs and Abiotic Stress-Related Hormones Abscissic Acid and Ethylene**

When plants encounter abiotic stress such as drought, ABA is synthesized to facilitate plant adaptation. Activated by ABA, the kinase SNF1-RELATED PROTEIN KINASE2 (SnRK2) phosphorylates the ABA-RESPONSIVE ELEMENTS-BINDING FACTORS (AREB/ABFs) TFs to promote transcription of ABA-responsive genes [98]. Similarly, osmotic stress also promotes the production of ethylene, which activates or stabilizes ETHYLENE INSENSITIVE (EIN) proteins of the ethylene signaling pathway. Two downstream TFs, EIN3 and EIN3-LIKE1 (EIL1) further induce numerous ETHYLENE RESPONSIVE FACTORS (ERFs) [5], which promote stress-responsive genes. Although the roles of ABA and ethylene in abiotic stress response are extensively studied, shedding light on the ABA/Et-controlled gene expression changes, it is unclear how and why ABA/Et also alter the levels of sRNAs in multiple species [99-107]. Excitingly, overexpression of some of these ABA-responsive sRNAs, like ath-miR168/393/394, renders arabidopsis more resistant to drought or salinity, suggesting that miRNAs are involved in the ABA-mediated drought response [108-110].

More than the other hormones discussed so far, ABA was reported to promote miRNA biogenesis through different pathways in arabidopsis (Figure 3). First, ABF transcription factors directly bind to the promoter of *MIR168A* for the induction of ath-miR168, the main miRNA targetting *AGO1* transcript for degradation. Conversely, mutating *AGO1* or overexpressing ath-miR168 results in ABA hypersensitivity and enhanced drought tolerance, suggesting that the miR168-AGO1 module is involved in

the ABA-dependent drought resistance [110]. Secondly, ABA stabilizes the CBP20/80 complex required for the stability of pre-miRNA transcripts (Box 1) [111]. This is, for example, the case for *ath-miR159*, which targets *MYB33/101*, encoding proteins required for ABA-mediated inhibition of seed germination (Figure 3). Accordingly, *cbp80* is hypersensitive to ABA, salt and osmotic stress during seed germination, which could in part be attributed to this *ath-miR159-MYB33/101* module [112]. Moreover, SE and HYL1 are phosphorylated by SnRK2, which promotes HYL1 abundance. Although the SE protein level is not altered, it was suggested that this phosphorylation might affect SE in its interaction with other factors [113]. Among the ABA-responsive miRNAs, *ath-miR842/846* forms another noteworthy example. Both miRNAs arise from the same functional isoform, with and without intron, respectively. ABA accumulation causes alternative splicing, resulting in accumulation of yet another isoform, thereby reducing *ath-miR842/846* [114]. However, the biological impact of this splicing-mediated sRNA control, is still unclear.

Besides ABA, ethylene also acts on CBP20 by promoting its phosphorylation, possibly re-inforcing its activity, resulting in upregulation of *ath-miR319* and downregulation of its target *MYB33*, but not *TCP2/4* in root (Figure 3). The *cbp20* mutant is less sensitive to ethylene, while the *MIR319b* overexpression line shows hypersensitivity to this hormone [115]. In turn, ethylene biosynthesis and salt tolerance genes are altered in *pvr-miR319*-overexpressing switchgrass (*Panicum virgatum*) upon ACC treatment, enabling enhanced salt-tolerance [116]. While no research reported direct induction of *MIR* transcription by ERFs, in petunia (*Petunia hybrida*), PhERF2 promotes *RDR2/6*, *DCL2* and *AGO1* expression to regulate siRNAs biogenesis and induce RNA silencing in response to viral infection [117] (Figure 3). Finally, ethylene and siRNAs are directly connected by *EIN5*, member of ethylene signaling pathway encoding a 5'-3' exoribonuclease necessary to enable aberrant transcript degradation. In *ein5* mutants, aberrant transcripts accumulate and generate siRNAs which induce post-transcriptional gene silencing (PTGS) [118,119].

ABA/Et biosynthesis or response are also regulated by miRNAs (Table 1). For example, in the case of the *gh-miR157-GhSPL10* module, overexpression of *GhSPL10* increases ethylene contents, promotes *ERF1/2* expression, and stimulates callus initiation in cotton (*Gossypium hirsutum*) [120]. Moreover, ethylene-mediated leaf senescence was reported to occur via EIN3-triggered repression of *ath-miR164*, which

targets *ORESARA/NAC2*, encoding a TF crucial for leaf senescence induction [121] (Figure 3). Finally, the kinase CONSTITUTIVE TRIPLE RESPONSE4 (SICTR4) of tomato can be silenced by sl-miR1917. As SICTR4 acts as a negative regulator of ethylene signaling, this silencing stimulates early fruit ripening [122]. On the ABA side, ABA induces ath-miR399f that targets *ABF3*, itself encoding a positive regulator of the ABA response, thereby creating a feedback loop [123] (Figure 3). Consistently, ath-miR399f overexpressing plants show a reduced sensitivity to ABA but decreased survival rate upon severe drought. Finally, ath-miR165/166 represses ABA response by targeting the TF *ABA INSENSITIVE4 (ABI4)* and also indirectly represses  $\beta$ -1,3-*GLUCANASE1 (BG1)*, an enzyme mediating ABA production. Therefore, miR165/166-defective mutant exhibits ABA- and drought hypersensitivity [124].

Overall, ABA/Et-regulated common phenotypic traits such as seed germination or leaf senescence can, at least in part, be attributed to sRNAs that affect the ABA/Et level and response [111,121]. Conversely, ABA/Et pathways also control sRNA levels by regulating core sRNAs biogenesis proteins, particularly CBP20, whose function is promoted by these hormones. This protein appears to have an important role in the ABA/Et response, but the precise mechanisms by which ABA/Et affect its phosphorylation and stability, are still unknown. Besides unraveling these molecular regulations, it would be interesting to investigate whether ABA/Et act on the same or distinct CBP20 phosphorylation site, by regulating a common or a specific kinase, respectively.

### **sRNAs Act as Crosstalk-Mediating Agents during Hormonal Communication**

Mutants in core sRNA regulators display hyper- or hyposensitivity to a range of hormones, as discussed earlier for the *hy1* mutant [21,37,44,75,77] (Box 1, Figure 4A). In addition, several miRNAs can be regulated by genes from more than one hormonal pathway (Figure 4B), and the same holds true for general sRNA-regulatory proteins like CPB20 that is regulated by the two abiotic stress hormones [111,112,115]. This suggests that sRNAs can act as hubs in hormonal networks, and two sRNAs in particular illustrate this.

First, miR159 levels are altered by BR, GA and ABA in different species [33,34,125]. In turn, miR159 is involved in the control of not less than 4 hormonal pathways: it promotes GA and BR biosynthesis, but, on the other hand, inhibits CK biosynthesis, and interferes with ABA in inhibition of seed germination. In the GA, BR and ABA

pathways, miR159 does so by targetting a TF of the MYB- or MYB-LIKE family [34,63,112,125,126]. Supporting a crucial role for miR159 in hormonal connections, suppression of *miR159* in arabidopsis and rice causes pleiotropic effects on plant growth that may be attributed to alteration of hormone levels [127,128].

Another clear example of miRNA contributing to the hormonal crosstalk is the miR156, the major orchestrator in age phase-transition [129]. miR156 inhibits GA or SL signal transduction by declining *SPL* expression. This miR156 thereby participates in, respectively, flowering time, branching, and JA-dependent insect defense [28,74,120]. Therefore, the miR156–*SPL* module may act as a hub for hormones in the regulation of diverse biological processes. More interestingly, these two crosstalking sRNAs also interact with each other, as deficiency in miR159 elevates miR156 levels, which leads to a delay of vegetative development [130]. Whether the miR159–miR156 balance is responsible for vegetative phase transition and its precise role in this process, is still elusive.

### **Concluding Remarks and Future Perspectives**

In the emerging sRNA-hormone network, it is clear that different steps of hormone biosynthesis can be affected by endogenous sRNAs, which is especially the case for GA, auxin, CK, and JA. By contrast, at the signal transduction level, the role of sRNAs seems to be more complex since either the sRNA regulators interplay with hormone responses or sRNA target genes belong to or participate in hormonal signaling. Conversely, hormones shape plant phenotypic plasticity under optimal and stress conditions and part of this regulation is achieved via sRNAs, as illustrated in the GA, Et, and ABA response. High-throughput sRNA-sequencing in different species in response to hormone treatment demonstrated that large sets of sRNAs are altered (Table 1). Hormones influence sRNAs biogenesis members and hormone-responsive factors regulate miRNA precursors. On the other hand, RSS proteins are also suggested to interfere with hormones, and thus offer another crucial link between sRNAs and hormones.

Although substantial advances have been achieved to understand the crosstalk between sRNAs and hormones, more research is required to elucidate several remaining questions (see Outstanding Questions). For example, some miRNAs and their targets, such as miR156-*SPL* and miR159-*GAMYB*, are evolutionarily conserved among species. Remarkably, the hormonal effect on these conserved

modules can be different between species, as illustrated by the promotion of miR159 in arabidopsis but not in rice. Therefore, it would be exciting to unravel the evolutionary basis underneath the hormonal, or even environmental, responses of miRNAs. On the other hand, newly identified miRNAs like os-miR444 and ath-miR842 were currently only described in one species. It would be interesting to identify the orthologous miRNAs based on these novel miRNAs' features. Additionally, small non-coding transfer-RNA derived fragments (tRFs) are emerging actors in the sRNA-hormone network: some are known to be stress- or ABA-responsive [131] or to modify auxin-regulatory enzymes [132], but what is the exact relationship between hormones and tRFs? Even regarding the above-described miRNAs and siRNAs, their precise mode-of-action in hormonal regulation is not completely clear. Finally, hormones can undergo long-distance transfer. Interestingly, sRNAs are able to transfer between cells, organisms, and species, for which they could be considered as "RNA hormones" [133,134]. Whether hormones and sRNAs would meet and regulate mutually in these crossroads forms another fascinating path for future research.

### **Acknowledgements**

We thank Dr. Annick Bleys for helpful suggestions for improving the manuscript. This work was supported by Ghent University [Bijzonder Onderzoeksfonds Methusalem Project BOF08/01M00408]. Marieke Dubois is a post-doctoral fellow of Flanders Research Foundation (FWO no. 12Q7919N).



## References

1. Benkova, E. (2016) Plant hormones in interactions with the environment. *Plant Mol. Biol.* 91, 597
2. Foo, E. *et al.* (2019) The Role of plant hormones in plant-microbe symbioses. *Front. Plant Sci.* 10
3. Zhu, J. and Geisler, M. (2015) Keeping it all together: auxin-actin crosstalk in plant development. *J. Exp. Bot.* 66, 4983-4998
4. Huang, H. *et al.* (2017) Jasmonate action in plant growth and development. *J. Exp. Bot.* 68, 1349-1359
5. Dubois, M. *et al.* (2018) The pivotal role of ethylene in plant growth. *Trends Plant Sci.* 23, 311-323
6. Blázquez, M.A. *et al.* (2020) Evolution of plant hormone response pathways. *Annu. Rev. Plant Biol.* 71
7. Ku, Y.-S. *et al.* (2018) Plant hormone signaling crosstalks between biotic and abiotic stress responses. *Int. J. Mol. Sci.* 19, 3206
8. Yang, J. *et al.* (2019) The crosstalks between jasmonic acid and other plant hormone signaling highlight the Involvement of Jasmonic Acid as a Core Component in Plant Response to Biotic and Abiotic Stresses. *Front. Plant Sci.* 10, 1349-1349
9. Li, N. *et al.* (2019) Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense: do we understand what they are whispering? *Int. J. Mol. Sci.* 20, 671
10. Tian, H. *et al.* (2017) Auxin-BR interaction regulates plant growth and development. *Front. Plant Sci.* 8, 2256
11. Ross, J.J. and Quittenden, L.J. (2016) Interactions between Brassinosteroids and Gibberellins: Synthesis or Signaling? *Plant Cell* 28, 829-832
12. Valluru, R. *et al.* (2016) Foliar abscisic acid-to-ethylene accumulation and response regulate shoot growth sensitivity to mild drought in wheat. *Front. Plant Sci.* 7, 461-461
13. Borges, F. and Martienssen, R.A. (2015) The expanding world of small RNAs in plants. *Nat. Rev. Mol. Cell Biol.* 16, 727-741
14. Robinson, D.O. and Roeder, A.H.K. (2017) Small RNAs turn over a new leaf as morphogens. *Dev. Cell* 43, 253-254
15. Singh, A. *et al.* (2018) Plant small RNAs: advancement in the understanding of biogenesis and role in plant development. *Planta* 248, 545-558
16. Koyama, T. *et al.* (2017) Roles of miR319 and TCP transcription factors in leaf development. *Plant Physiol.* 175, 874-885
17. Liebsch, D. and Palatnik, J.F. (2020) MicroRNA miR396, GRF transcription factors and GIF co-regulators: a conserved plant growth regulatory module with potential for breeding and biotechnology. *Curr. Opin. Plant Biol.* 53, 31-42
18. Deng, Y. *et al.* (2018) A role for small RNA in regulating innate immunity during plant growth. *PLoS Pathog.* 14, e1006756-e1006756
19. Prasad, A. *et al.* (2019) Recent advances in small RNA mediated plant-virus interactions. *Crit. Rev. Biotechnol.* 39, 587-601
20. Li, S. *et al.* (2017) The functions of plant small RNAs in development and in stress responses. *Plant J.* 90, 654-670
21. Song, X. *et al.* (2019) MicroRNAs and their regulatory roles in plant-environment interactions. *Annu. Rev. Plant Biol.* 70, 489-525
22. Hedden, P. and Sponsel, V. (2015) A century of gibberellin research. *J. Plant Growth Regul.* 34, 740-760
23. Han, J. *et al.* (2014) Grapevine microRNAs responsive to exogenous gibberellin. *BMC Genomics* 15, 111

24. Fan, S. *et al.* (2018) Mediation of flower induction by gibberellin and its inhibitor paclobutrazol: mRNA and miRNA integration comprises complex regulatory cross-talk in apple. *Plant Cell Physiol.* 59, 2288-2307
25. Lu, Y. *et al.* (2020) SLENDER RICE1 and oryza sativa INDETERMINATE DOMAIN2 regulating OsmiR396 is involved in stem elongation. *Plant Physiol.*
26. Li, K. *et al.* (2016) DELLA-mediated PIF degradation contributes to coordination of light and gibberellin signalling in Arabidopsis. *Nat. Commun.* 7, 1-11
27. Sun, Z. *et al.* (2018) Coordinated regulation of *Arabidopsis* microRNA biogenesis and red light signaling through Dicer-like 1 and phytochrome-interacting factor 4. *PLoS Genet.* 14, e1007247
28. Yu, S. *et al.* (2012) Gibberellin regulates the *Arabidopsis* floral transition through miR156-targeted SQUAMOSA PROMOTER BINDING-LIKE transcription factors. *Plant Cell* 24, 3320-3332
29. Ma, Z. *et al.* (2014) *Arabidopsis* miR171-targeted scarecrow-like proteins bind to GT *cis*-elements and mediate gibberellin-regulated chlorophyll biosynthesis under light conditions. *PLoS Genet.* 10, e1004519
30. Huang, W. *et al.* (2017) Overexpression of a tomato miR171 target gene *SIGRAS24* impacts multiple agronomical traits via regulating gibberellin and auxin homeostasis. *Plant Biotechnol. J.* 15, 472-488
31. Guo, G. *et al.* (2018) Wheat miR9678 affects seed germination by generating phased siRNAs and modulating abscisic acid/gibberellin signaling. *Plant Cell* 30, 796-814
32. Millar, A.A. *et al.* (2019) Biology and function of miR159 in plants. *Plants* 8, 255
33. Achard, P. *et al.* (2004) Modulation of floral development by a gibberellin-regulated microRNA. *Development* 131, 3357-3365
34. Gao, J. *et al.* (2018) A brassinosteroid responsive miRNA - target module regulates gibberellin biosynthesis and plant development. *New Phytol.* 220, 488-501
35. Ackerman-Lavert, M. and Savaldi-Goldstein, S. (2020) Growth models from a brassinosteroid perspective. *Curr. Opin. Plant Biol.* 53, 90-97
36. Sirohi, G. *et al.* (2019) High-throughput sequencing and differential expression analysis of miRNAs in response to brassinosteroid treatment in *Arabidopsis thaliana*. *Funct. Integr. Genomics* 19, 597-615
37. Wei, L. *et al.* (2014) Dicer-like 3 produces transposable element-associated 24-nt siRNAs that control agricultural traits in rice. *Proc. Natl. Acad. Sci. USA* 111, 3877-3882
38. Xia, K. *et al.* (2015) Rice microRNA osa-miR1848 targets the obtusifoliol 14 $\alpha$ -demethylase gene OsCYP51G3 and mediates the biosynthesis of phytosterols and brassinosteroids during development and in response to stress. *New Phytol.* 208, 790-802
39. Zhang, Y.-C. *et al.* (2013) Overexpression of microRNA OsmiR397 improves rice yield by increasing grain size and promoting panicle branching. *Nat. Biotechnol.* 31, 848-852
40. Jiao, X. *et al.* (2019) Promotion of BR biosynthesis by miR444 is required for ammonium-triggered inhibition of root growth. *Plant Physiol.*
41. Tang, Y. *et al.* (2018) OsmiR396d affects gibberellin and brassinosteroid signaling to regulate plant architecture in rice. *Plant Physiol.* 176, 946-959
42. Enders, T.A. and Strader, L.C. (2015) Auxin activity: past, present, and future. *Am. J. Bot.* 102, 180-196
43. Sorin, C. *et al.* (2005) Auxin and light control of adventitious rooting in Arabidopsis require ARGONAUTE1. *Plant Cell* 17, 1343-1359
44. Lu, C. and Fedoroff, N. (2000) A mutation in the Arabidopsis *HYL1* gene encoding a dsRNA binding protein affects responses to abscisic acid, auxin, and cytokinin. *Plant Cell* 12, 2351-2365
45. Kong, W. *et al.* (2015) A novel Arabidopsis microRNA promotes IAA biosynthesis via the indole-3-acetaldoxime pathway by suppressing *SUPERROOT1*. *Plant Cell Physiol.* 56, 715-726
46. Merelo, P. *et al.* (2017) The shady side of leaf development: the role of the REVOLUTA/KANADI1 module in leaf patterning and auxin-mediated growth promotion. *Curr. Opin. Plant Biol.* 35, 111-116

47. Zhao, Y.F. *et al.* (2019) miR1432 - OsACOT (Acyl - CoA thioesterase) module determines grain yield via enhancing grain filling rate in rice. *Plant Biotechnol. J.* 17, 712-723
48. Kinoshita, N. *et al.* (2012) IAA-Ala Resistant3, an evolutionarily conserved target of miR167, mediates Arabidopsis root architecture changes during high osmotic stress. *Plant Cell* 24, 3590-3602
49. Gyula, P. *et al.* (2018) Ambient temperature regulates the expression of a small set of sRNAs influencing plant development through NF - YA2 and YUC2. *Plant Cell Environ.* 41, 2404-2417
50. Si-Ammour, A. *et al.* (2011) miR393 and secondary siRNAs regulate expression of the TIR1/AFB2 auxin receptor clade and auxin-related development of Arabidopsis leaves. *Plant Physiol.* 157, 683-691
51. Wang, J.-J. and Guo, H.-S. (2015) Cleavage of INDOLE-3-ACETIC ACID INDUCIBLE28 mRNA by microRNA847 upregulates auxin signaling to modulate cell proliferation and lateral organ growth in Arabidopsis. *Plant Cell* 27, 574-590
52. Liu, N. *et al.* (2014) Down-regulation of AUXIN RESPONSE FACTORS 6 and 8 by microRNA 167 leads to floral development defects and female sterility in tomato. *J. Exp. Bot.* 65, 2507-2520
53. Mallory, A.C. *et al.* (2005) MicroRNA-directed regulation of Arabidopsis AUXIN RESPONSE FACTOR17 is essential for proper development and modulates expression of early auxin response genes. *Plant Cell* 17, 1360-1375
54. Roodbarkelari, F. *et al.* (2015) ZLL/AGO10 maintains shoot meristem stem cells during Arabidopsis embryogenesis by down-regulating ARF2-mediated auxin response. *BMC Biol.* 13, 74-74
55. Barik, S. *et al.* (2015) Coevolution pattern and functional conservation or divergence of miR167s and their targets across diverse plant species. *Sci. Rep.* 5, 14611
56. Müller, C.J. *et al.* (2016) PHABULOSA mediates an auxin signaling loop to regulate vascular patterning in Arabidopsis. *Plant Physiol.* 170, 956-970
57. Dastidar, M.G. *et al.* (2019) ARF5/MONOPTEROS directly regulates miR390 expression in the Arabidopsis thaliana primary root meristem. *Plant Direct* 3, e00116-e00116
58. Marin, E. *et al.* (2010) miR390, Arabidopsis TAS3 tasiRNAs, and their AUXIN RESPONSE FACTOR targets define an autoregulatory network quantitatively regulating lateral root growth. *Plant Cell* 22, 1104-1117
59. Skopelitis, D.S. *et al.* (2017) Boundary formation through a direct threshold-based readout of mobile small RNA gradients. *Dev. Cell* 43, 265-273.e266
60. Kieber, J.J. and Schaller, G.E. (2018) Cytokinin signaling in plant development. *Development* 145, dev149344
61. Bhogale, S. *et al.* (2014) MicroRNA156: a potential graft-transmissible microRNA that modulates plant architecture and tuberization in Solanum tuberosum ssp. andigena. *Plant Physiol.* 164, 1011-1027
62. Scofield, S. *et al.* (2014) STM sustains stem cell function in the Arabidopsis shoot apical meristem and controls KNOX gene expression independently of the transcriptional repressor AS1. *Plant Signal. Behav.* 9, e28934
63. Rubio-Somoza, I. and Weigel, D. (2013) Coordination of flower maturation by a regulatory circuit of three microRNAs. *PLoS Genet.* 9, e1003374
64. Zhang, Y. *et al.* (2020) A novel microRNA, SlymiR208, promotes leaf senescence via regulating cytokinin biosynthesis in tomato. *Physiol. Plant.*
65. Zubko, E. and Meyer, P. (2007) A natural antisense transcript of the *Petunia hybrida* Sho gene suggests a role for an antisense mechanism in cytokinin regulation. *Plant J.* 52, 1131-1139
66. Zhang, T.-Q. *et al.* (2015) An intrinsic microRNA timer regulates progressive decline in shoot regenerative capacity in plants. *Plant Cell* 27, 349-360
67. Dello Iorio, R. *et al.* (2012) A PHABULOSA/cytokinin feedback loop controls root growth in Arabidopsis. *Curr. Biol.* 22, 1699-1704
68. Liu, Z. *et al.* (2016) Repression of callus initiation by the mi RNA - directed interaction of auxin-cytokinin in Arabidopsis thaliana. *Plant J.* 87, 391-402

69. Turner, M. *et al.* (2013) Ectopic expression of miR160 results in auxin hypersensitivity, cytokinin hyposensitivity, and inhibition of symbiotic nodule development in soybean. *Plant Physiol.* 162, 2042-2055
70. Ariel, F. *et al.* (2012) Two direct targets of cytokinin signaling regulate symbiotic nodulation in *Medicago truncatula*. *Plant Cell* 24, 3838-3852
71. Hofferek, V. *et al.* (2014) MiR171h restricts root symbioses and shows like its target NSP2 a complex transcriptional regulation in *Medicago truncatula*. *BMC Plant Biol.* 14, 199-199
72. Waters, M.T. *et al.* (2017) Strigolactone signaling and evolution. *Annu. Rev. Plant Biol.* 68, 291-322
73. Chen, Z. *et al.* (2015) Alteration of *osa-miR156e* expression affects rice plant architecture and strigolactones (SLs) pathway. *Plant Cell Rep.* 34, 767-781
74. Song, X. *et al.* (2017) IPA1 functions as a downstream transcription factor repressed by D53 in strigolactone signaling in rice. *Cell Res.* 27, 1128-1141
75. Wu, Y.Y. *et al.* (2017) DCL2 - and RDR6 - dependent transitive silencing of *SMXL4* and *SMXL5* in *Arabidopsis dcl4* mutants causes defective phloem transport and carbohydrate over - accumulation. *Plant J.* 90, 1064-1078
76. Tsuda, K. and Somssich, I.E. (2015) Transcriptional networks in plant immunity. *New Phytol.* 206, 932-947
77. Kwon, T. (2016) A double-stranded RNA binding protein, HYL1, regulates plant immunity via the jasmonic acid pathway. *J. Plant Biol.* 59, 506-514
78. López, A. *et al.* (2011) The RNA silencing enzyme RNA polymerase V is required for plant immunity. *PLoS Genet.* 7, e1002434
79. López Sánchez, A. *et al.* (2016) The role of DNA (de)methylation in immune responsiveness of *Arabidopsis*. *Plant J.* 88, 361-374
80. Zhang, Y. and Li, X. (2019) Salicylic acid: biosynthesis, perception, and contributions to plant immunity. *Curr. Opin. Plant Biol.* 50, 29-36
81. Alazem, M. *et al.* (2019) Effects of abscisic acid and salicylic acid on gene expression in the antiviral RNA silencing pathway in *Arabidopsis*. *Int. J. Mol. Sci.* 20, 2538
82. Chen, L. *et al.* (2017) MicroRNA396a-5p and -3p induce tomato disease susceptibility by suppressing target genes and upregulating salicylic acid. *Plant Sci.* 265, 177-187
83. Han, G.-Z. (2017) Evolution of jasmonate biosynthesis and signaling mechanisms. *J. Exp. Bot.* 68, 1323-1331
84. Liu, C. *et al.* (2018) *Arabidopsis* ARGONAUTE 1 binds chromatin to promote gene transcription in response to hormones and stresses. *Dev. Cell* 44, 348-361
85. Zhang, X.-C. *et al.* (2015) Jasmonate signalling in *Arabidopsis* involves SGT1b-HSP70-HSP90 chaperone complexes. *Nat. Plants* 1, 15049
86. Zhang, C. *et al.* (2016) Suppression of jasmonic acid-mediated defense by viral-inducible microRNA319 facilitates virus infection in rice. *Mol. Plant* 9, 1302-1314
87. Schommer, C. *et al.* (2008) Control of jasmonate biosynthesis and senescence by miR319 targets. *PLoS Biol.* 6, e230
88. Zhao, W. *et al.* (2015) Identification of jasmonic acid-associated microRNAs and characterization of the regulatory roles of the miR319/TCP4 module under root-knot nematode stress in tomato. *J. Exp. Bot.* 66, 4653-4667
89. Mao, Y.-B. *et al.* (2017) Jasmonate response decay and defense metabolite accumulation contributes to age-regulated dynamics of plant insect resistance. *Nat. Commun.* 8, 13925
90. De Vleeschauwer, D. *et al.* (2018) Target of rapamycin signaling orchestrates growth-defense trade-offs in plants. *New Phytol.* 217, 305-319
91. Zvereva, A.S. *et al.* (2016) Viral protein suppresses oxidative burst and salicylic acid-dependent autophagy and facilitates bacterial growth on virus-infected plants. *New Phytol.* 211, 1020-1034
92. Poque, S. *et al.* (2018) Potyviral gene-silencing suppressor HCPro interacts with salicylic acid (SA)-binding protein 3 to weaken SA-mediated defense responses. *Mol. Plant-Microbe Interact.* 31, 86-100

93. Soitamo, A.J. *et al.* (2012) Expression of geminiviral AC2 RNA silencing suppressor changes sugar and jasmonate responsive gene expression in transgenic tobacco plants. *BMC Plant Biol.* 12, 204
94. Haikonen, T. *et al.* (2013) Mutation of a short variable region in HCpro protein of *Potato virus A* affects interactions with a microtubule-associated protein and induces necrotic responses in tobacco. *Mol. Plant-Microbe Interact.* 26, 721-733
95. Westwood, J.H. *et al.* (2014) Interference with jasmonic acid-regulated gene expression is a general property of viral suppressors of RNA silencing but only partly explains virus-induced changes in plant-aphid interactions. *J. Gen. Virol.* 95, 733-739
96. Rosas-Díaz, T. *et al.* (2016) The C2 protein from the geminivirus *Tomato yellow leaf curl Sardinia virus* decreases sensitivity to jasmonates and suppresses jasmonate-mediated defences. *Plants* 5, 8
97. Zhang, B. *et al.* (2012) Global analysis of non-coding small RNAs in Arabidopsis in response to jasmonate treatment by deep sequencing technology. *J. Integr. Plant Biol.* 54, 73-86
98. Chen, K. *et al.* (2020) Absciscic acid dynamics, signaling, and functions in plants. *J. Integr. Plant Biol.* 62, 25-54
99. Wang, Y. *et al.* (2017) Parsing the regulatory network between small RNAs and target genes in ethylene pathway in tomato. *Front. Plant Sci.* 8, 527
100. Zhao, F. *et al.* (2017) Characterization of miRNAs responsive to exogenous ethylene in grapevine berries at whole genome level. *Funct. Integr. Genomics* 17, 213-235
101. Dan, M. *et al.* (2018) Identification of ethylene responsive miRNAs and their targets from newly harvested banana fruits using high-throughput sequencing. *J. Agric. Food Chem.* 66, 10628-10639
102. Chen, L. *et al.* (2012) Ethylene-responsive miRNAs in roots of *Medicago truncatula* identified by high-throughput sequencing at whole genome level. *Plant Sci.* 184, 14-19
103. Zuo, J. *et al.* (2012) Sculpting the maturation, softening and ethylene pathway: the influences of microRNAs on tomato fruits. *BMC Genomics* 13, 7
104. Duan, H. *et al.* (2016) Genome-wide analysis of microRNA responses to the phytohormone abscisic acid in *Populus euphratica*. *Front. Plant Sci.* 7, 1184
105. Tian, C. *et al.* (2015) Identification and characterization of ABA-responsive microRNAs in rice. *J. Genet.* 42, 393-402
106. Li, D. *et al.* (2019) Integrated analysis of high-throughput sequencing data shows abscisic acid-responsive genes and miRNAs in strawberry receptacle fruit ripening. *Hortic. Res.* 6, 1-13
107. Cheng, H.-Y. *et al.* (2016) Genomic profiling of exogenous abscisic acid-responsive microRNAs in tomato (*Solanum lycopersicum*). *BMC Genomics* 17, 423
108. Song, J.B. *et al.* (2013) miR394 and LCR are involved in Arabidopsis salt and drought stress responses in an abscisic acid-dependent manner. *BMC Plant Biol.* 13, 210
109. Long, R. *et al.* (2017) A novel miRNA sponge form efficiently inhibits the activity of miR393 and enhances the salt tolerance and ABA insensitivity in *Arabidopsis thaliana*. *Plant Mol. Biol. Rep.* 35, 409-415
110. Li, W. *et al.* (2012) Transcriptional regulation of Arabidopsis *MIR168a* and *ARGONAUTE1* homeostasis in abscisic acid and abiotic stress responses. *Plant Physiol.* 158, 1279-1292
111. Kim, S. *et al.* (2008) Two cap-binding proteins CBP20 and CBP80 are involved in processing primary MicroRNAs. *Plant Cell Physiol.* 49, 1634-1644
112. Daszkowska-Golec, A. (2018) Emerging roles of the nuclear cap-binding complex in abiotic stress responses. *Plant Physiol.* 176, 242-253
113. Yan, J. *et al.* (2017) The SnRK2 kinases modulate miRNA accumulation in *Arabidopsis*. *PLoS Genet.* 13, e1006753
114. Jia, F. and Rock, C.D. (2013) *MIR846* and *MIR842* comprise a cistronic *MIRNA* pair that is regulated by abscisic acid by alternative splicing in roots of Arabidopsis. *Plant Mol. Biol.* 81, 447-460
115. Zhang, F. *et al.* (2016) Phosphorylation of CBP20 links microRNA to root growth in the ethylene response. *PLoS Genet.* 12, e1006437
116. Liu, Y. *et al.* (2019) MiR319 mediated salt tolerance by ethylene. *Plant Biotechnol. J.* 17, 2370-2383

117. Sun, D. *et al.* (2016) A petunia ethylene-responsive element binding factor, *PhERF2*, plays an important role in antiviral RNA silencing. *J. Exp. Bot.* 67, 3353-3365
118. Zhang, X. *et al.* (2015) Suppression of endogenous gene silencing by bidirectional cytoplasmic RNA decay in *Arabidopsis*. *Science* 348, 120-123
119. Li, T. *et al.* (2019) A genetics screen highlights emerging roles for CPL3, RST1 and URT1 in RNA metabolism and silencing. *Nat. Plants* 5, 539-550
120. Wang, L. *et al.* (2018) The *GhmiR157a-GhSPL10* regulatory module controls initial cellular dedifferentiation and callus proliferation in cotton by modulating ethylene-mediated flavonoid biosynthesis. *J. Exp. Bot.* 69, 1081-1093
121. Li, Z. *et al.* (2013) *ETHYLENE-INSENSITIVE3* is a senescence-associated gene that accelerates age-dependent leaf senescence by directly repressing *miR164* transcription in *Arabidopsis*. *Plant Cell* 25, 3311-3328
122. Wang, Y. *et al.* (2018) MicroRNA1917 targets CTR4 splice variants to regulate ethylene responses in tomato. *J. Exp. Bot.* 69, 1011-1025
123. Baek, D. *et al.* (2016) A role for *Arabidopsis miR399f* in salt, drought, and ABA signaling. *Mol. Cells* 39, 111-118
124. Yan, J. *et al.* (2016) The miR165/166 mediated regulatory module plays critical roles in ABA homeostasis and response in *Arabidopsis thaliana*. *PLoS Genet.* 12, e1006416
125. Reyes, J.L. and Chua, N.-H. (2007) ABA induction of miR159 controls transcript levels of two MYB factors during *Arabidopsis* seed germination. *Plant J.* 49, 592-606
126. da Silva, E.M. *et al.* (2017) MicroRNA159 - targeted *SIGAMYB* transcription factors are required for fruit set in tomato. *Plant J.* 92, 95-109
127. Zhao, Y. *et al.* (2017) Suppression of microRNA159 impacts multiple agronomic traits in rice (*Oryza sativa* L.). *BMC Plant Biol.* 17, 215
128. Alonso-Peral, M.M. *et al.* (2010) The microRNA159-regulated GAMYB-like genes inhibit growth and promote programmed cell death in *Arabidopsis*. *Plant Physiol.* 154, 757-771
129. Wang, J.-W. (2016) The multifaceted roles of miR156-targeted SPL transcription factors in plant developmental transitions. In *Plant Transcription Factors*, pp. 281-293, Elsevier
130. Guo, C. *et al.* (2017) Repression of miR156 by miR159 regulates the timing of the juvenile-to-adult transition in *Arabidopsis*. *Plant Cell* 29, 1293-1304
131. Park, E.J. and Kim, T.-H. (2018) Fine-tuning of gene expression by tRNA-derived fragments during abiotic stress signal transduction. *Int. J. Mol. Sci.* 19, 518
132. Soprano, A.S. *et al.* (2018) Regulation of tRNA biogenesis in plants and its link to plant growth and response to pathogens. *Biochim. Biophys. Acta - Gene Regul. Mech.* 1861, 344-353
133. Bayraktar, R. *et al.* (2017) Cell-to-cell communication: microRNAs as hormones. *Mol. Oncol.* 11, 1673-1686
134. Cai, Q. *et al.* (2018) Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Science* 360, 1126-1129
135. Zhao, T. *et al.* (2019) Identification and profiling of upland cotton microRNAs at fiber initiation stage under exogenous IAA application. *BMC Genomics* 20, 421
136. Li, Q. *et al.* (2017) Identification of novel miRNAs and miRNA expression profiling in embryogenic tissues of *Picea balfouriana* treated by 6-benzylaminopurine. *PLoS ONE* 12
137. Ma, C. *et al.* (2019) Differential expression of the microRNAs are responsive to drought stress and exogenous methyl jasmonate in wheat (*Triticum aestivum*). *Int. J. Agr. Biol.* 22, 475-486
138. Nazaruddin, N. *et al.* (2017) Small RNA-seq analysis in response to methyl jasmonate and abscisic acid treatment in *Persicaria minor*. *Genomics data* 12, 157-158
139. Bologna, N.G. *et al.* (2018) Nucleo-cytosolic shuttling of ARGONAUTE1 prompts a revised model of the plant microRNA pathway. *Mol. Cell* 69, 709-719
140. Xie, M. and Yu, B. (2015) siRNA-directed DNA methylation in plants. *Curr. Genomics* 16, 23-31
141. Megel, C. *et al.* (2018) Plant RNases T2, but not Dicer-like proteins, are major players of tRNA-derived fragments biogenesis. *Nucleic Acids Res.* 47, 941-952

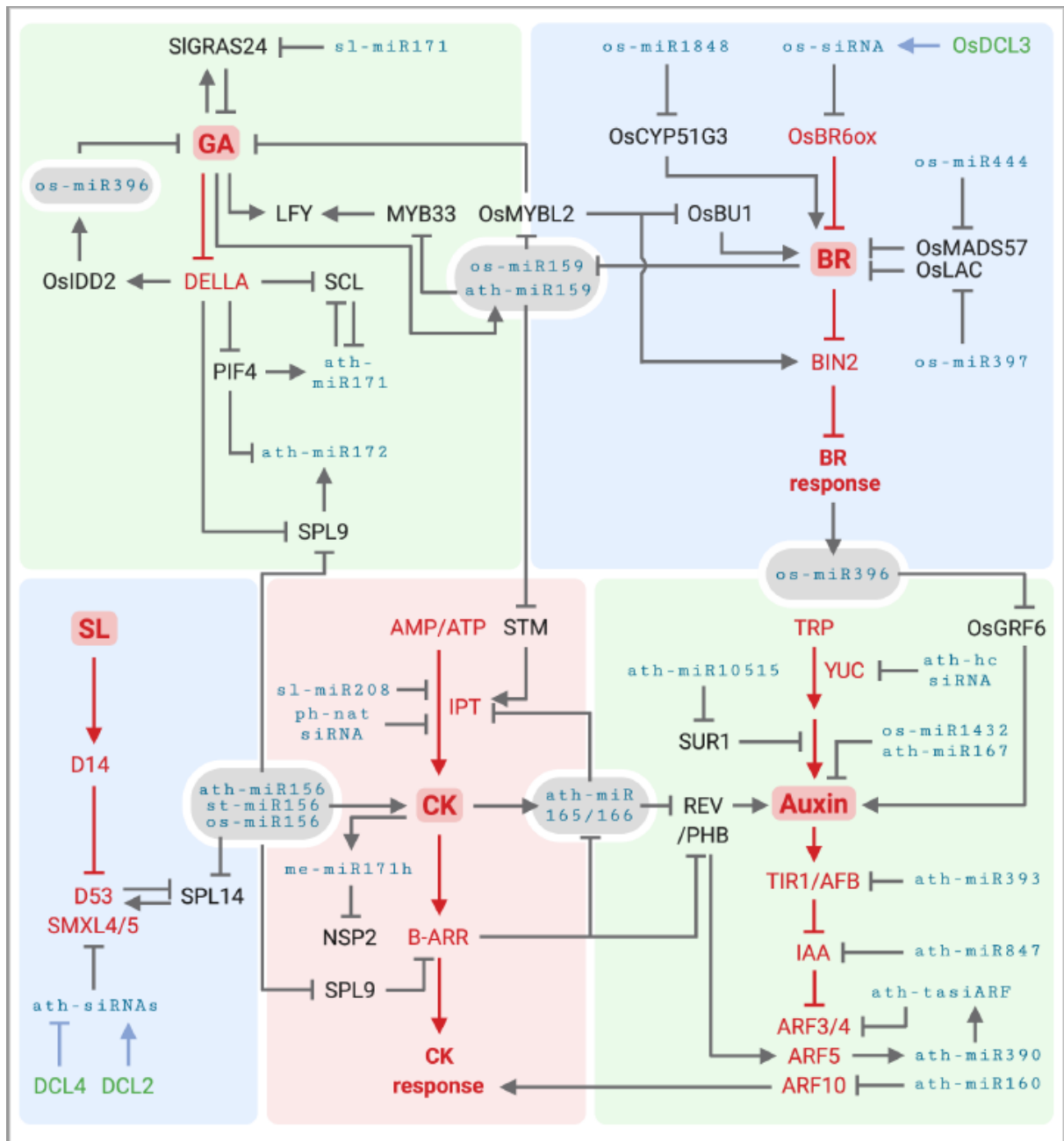
142. Alves, C.S. *et al.* (2017) Genome-wide identification and characterization of tRNA-derived RNA fragments in land plants. *Plant Mol. Biol.* 93, 35-48
143. Loss-Morais, G. *et al.* (2013) Description of plant tRNA-derived RNA fragments (tRFs) associated with argonaute and identification of their putative targets. *Biol. Direct* 8, 6
144. Cao, M. *et al.* (2014) Virus infection triggers widespread silencing of host genes by a distinct class of endogenous siRNAs in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 111, 14613-14618
145. Schuck, J. *et al.* (2013) AGO/RISC-mediated antiviral RNA silencing in a plant *in vitro* system. *Nucleic Acids Res.* 41, 5090-5103
146. Raja, P. *et al.* (2014) *Arabidopsis* double-stranded RNA binding protein DRB3 participates in methylation-mediated defense against geminiviruses. *J. Virol.* 88, 2611-2622
147. Michaeli, S. *et al.* (2019) The viral F-box protein P0 induces an ER-derived autophagy degradation pathway for the clearance of membrane-bound AGO1. *Proc. Natl. Acad. Sci. USA* 116, 22872-22883
148. Burguán, J. and Havelda, Z. (2011) Viral suppressors of RNA silencing. *Trends Plant Sci.* 16, 265-272

### **Outstanding Questions**

- What are the more precise molecular mechanisms underlying hormone-mediated regulation of sRNA?
- How do SLs and recently identified tRFs integrate in the sRNAs–hormone network?
- Regarding the mobility of sRNAs and hormones in plants, are hormone-related proteins participating in the regulation of sRNA trafficking, thereby indirectly influencing gene silencing?

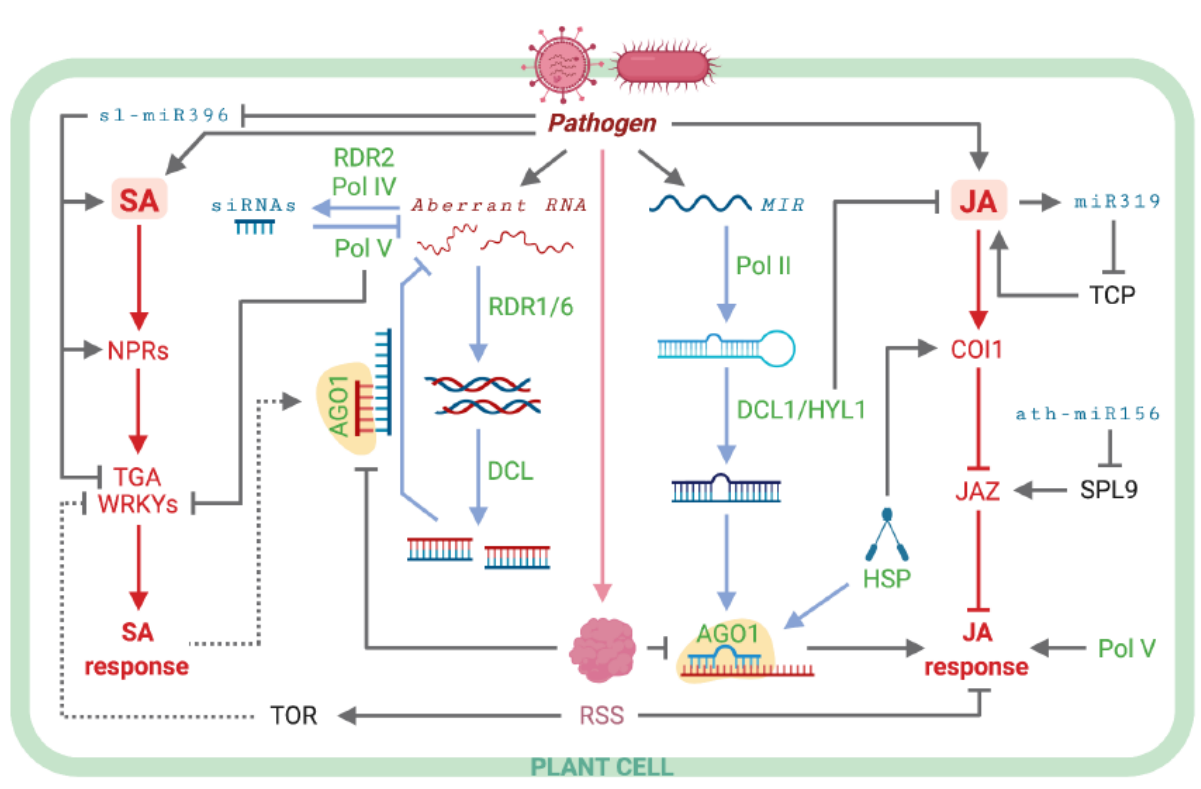


## FIGURES

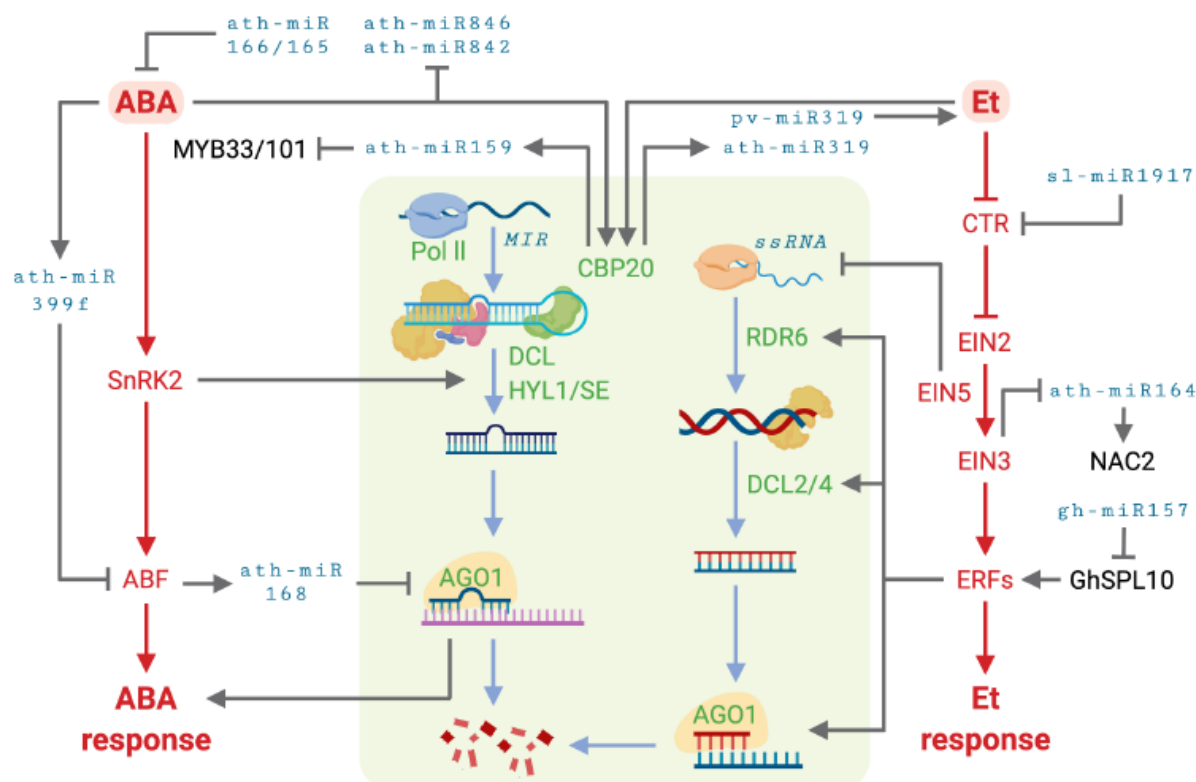


**Figure 1. Molecular network connecting sRNAs and the growth-regulatory hormones.** During plant development, gibberellic acid (GA), auxin, cytokinins (CK), brassinosteroids (BR) and strigolactones (SL) control multiple aspects of plant growth, particularly cell division and differentiation. Hundreds of sRNAs are responsive to these hormones (Table 1), and multiple miRNAs (blue) participate in the fine-tuning of hormone biosynthesis or signaling (red). Because some miRNAs are responsive to a subset of hormones and in turn regulate other hormones, they could form new connections in hormonal networks (grey modules). Abbreviations: GROWTH-REGULATING FACTOR (GRF), SCARECROW-LIKE27 (SCL27), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), INDETERMINATE DOMAIN2 (IDD2), PHYTOCHROME INTERACTING FACTOR 4 (PIF4), LEAFY (LFY), DICER-LIKE (DCL), BRASSINOSTEROID-INSENSITIVE2 (BIN2), LACCASE (LAC), BRASSINOSTEROID UPREGULATED1, BRASSINOSTEROID-6-OXIDASE (BR6ox),

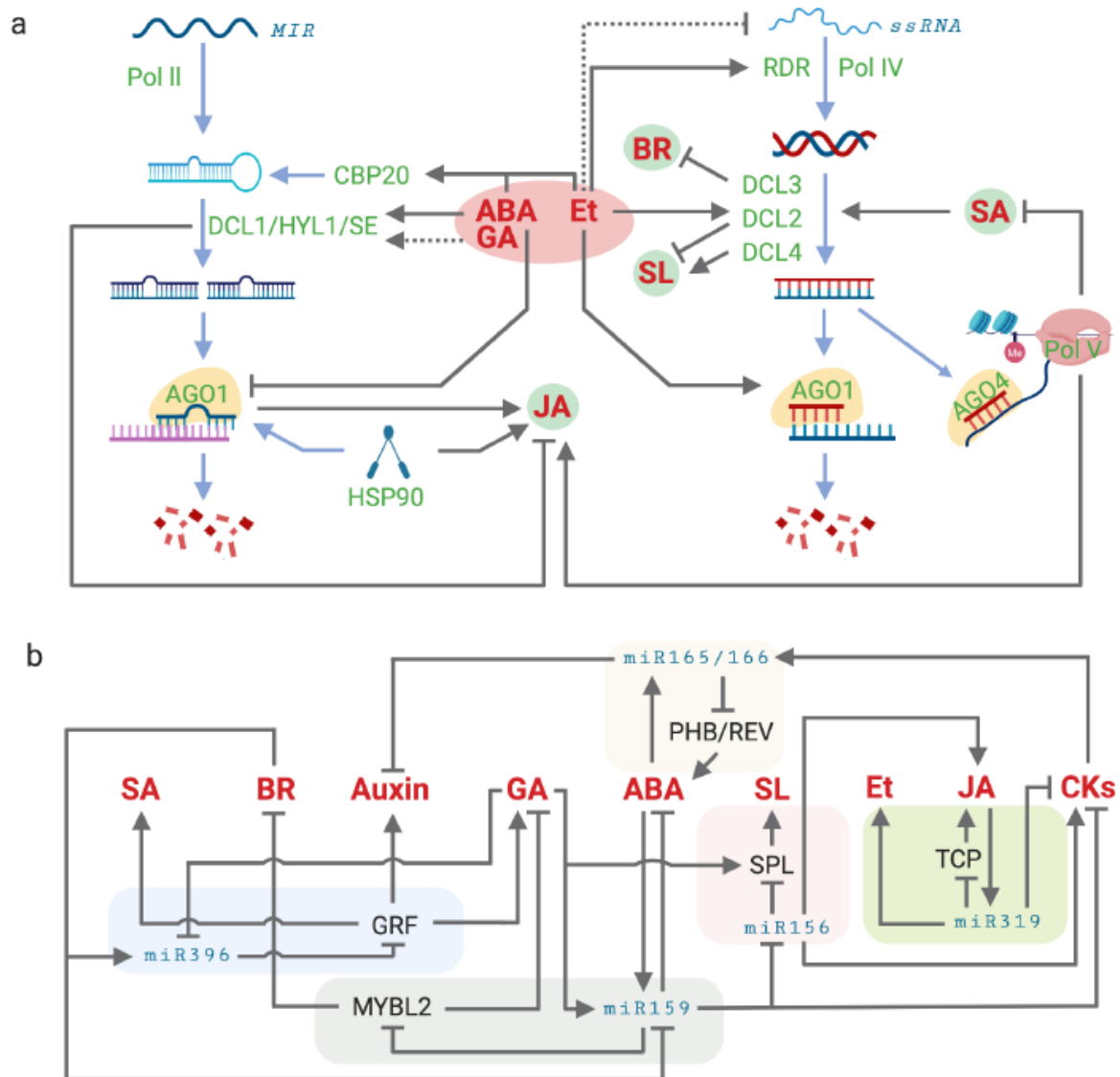
AUXIN RESPONSE FACTOR (ARF), YUCCA (YUC), SUPERROOT1 (SUR1), TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB), INDOLE-3-ACETIC ACID INDUCIBLE (IAA), ARABIDOPSIS RESPONSE REGULATORS (ARRs), PHABULOSA/REVOLUTA (PHB/REV), SHOOTMERISTEMLESS (STM), NODULATION SIGNALING PATHWAY2 (NSP2), DWARF14 (D14), SUPPRESSOR OF MAX2 1-LIKE (SMXL), ISOPENTENYLTRANSFERASE (IPT), Tryptophan (Trp), Adenosine monophosphate/Adenosine triphosphate (AMP/ATP), *Oryza sativa* (os), *Solanum lycopersicum* (sl), *Medicago truncatula* (me), *Arabidopsis thaliana* (ath) *Solanum tuberosum* (st).



**Figure 2. Regulation of the biotic stress-induced hormones salicylic acid (SA) and jasmonate (JA) by sRNAs.** Upon viral or bacterial infection, plants stimulate biosynthesis of SA or JA depending on the pathogen. Key proteins of sRNA biogenesis or function (green) can regulate SA- or JA-biosynthesis genes and downstream transcription factors (red), a mechanism that is hijacked by pathogens. Additionally, viruses produce RNA silencing suppressor (RSS) proteins that repress the plant's ARGONAUTE 1 (AGO1) proteins, central players in post-transcriptional regulation of defense genes. Finally, sRNA species (blue) act directly on SA- or JA-biosynthesis or signaling genes, by either promoting or inhibiting it. Abbreviations: RNA-DEPENDENT RNA POLYMERASE (RDR), RNA polymerase (Pol), NONEXPRESSER OF PR-GENES (NPR), TGACGTCA CIS-ELEMENT-BINDING PROTEIN (TGA), DICER-LIKE (DCL), HYPOONASTIC LEAVES1 (HYL1), CORONATINE INSENSITIVE1 (COI1), JASMONATE-ZIM-DOMAIN PROTEIN (JAZ), HEAT SHOCK PROTEIN (HSP), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), *Solanum lycopersicum* (sl), *Arabidopsis thaliana* (ath).



**Figure 3. Interconnection of abiotic stress-responsive hormones ABA and ethylene with miRNAs.** When plants experience abiotic stress, abscisic acid (ABA) and ethylene (Et) are rapidly synthesized. The biosynthesis of these hormones (red) is regulated by miRNAs (blue), themselves affected by the hormone in a feedback mechanism. Other hormone-induced miRNAs can fine-tune the downstream signaling pathway of ABA and Et (red), respectively. Finally, key effectors in sRNA biogenesis or function (green) can be controlled by ABA, Et, or their respective downstream transcription factor. Abbreviations: SNF1-RELATED PROTEIN KINASE2 (SnRK2), ETHYLENE INSENSITIVE (EIN), ETHYLENE RESPONSIVE FACTOR (ERF), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), ABRE BINDING FACTOR (ABF), DICER-LIKE (DCL), HYPONASTIC LEAVES1 (HYL1), ARGONAUTE1 (AGO1), RNA-DEPENDENT RNA POLYMERASE (RDR), RNA polymerase (Pol), CAPING BINDING PROTEIN (CBP), *Gossypium hirsutum* (gh), *Panicum virgatum* (pv), *Arabidopsis thaliana* (ath).



**Figure 4. sRNA-modules as novel hubs in hormonal crosstalk.** (A) Central proteins in sRNA biogenesis or function (green) can be induced by multiple hormones, including abscisic acid (ABA), ethylene (Et), and salicylic acid (SA). In turn, they can affect strigolactones (SL), brassinosteroids (BR) or jasmonate (JA). Their regulation by hormones and their capacity to, in turn, fine-tune other hormonal responses, places them as new possible hubs in plant hormonal crosstalks. (B) Multiple miRNA species can form new bridges in hormonal crosstalk. miR156, miR159, miR165/166, miR319, and miR396 are regulated by ABA, BR, JA, or gibberellic acid (GA) and in turn participate in the control of SA, auxin, cytokinin (CK) or SL, thereby providing miRNA-regulated connections between hormones. Abbreviations: PHABULOSA/REVOLUTA (PHB/REV), GROWTH-REGULATING FACTOR (GRF), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), DICER-LIKE (DCL), HYPOPLASTIC LEAVES1 (HYL1), ARGONAUTE1 (AGO1), RNA-DEPENDENT RNA POLYMERASE (RDR), RNA polymerase (Pol), CAPING BINDING PROTEIN (CBP), SERRATE (SE), HEAT SHOCK PROTEIN (HSP).

## TABLES

**Table 1. Overview of the important sRNA modules in hormonal responses<sup>a</sup>**

Hormones	Genome-wide analysis	Involvement	Key miRNAs	Target	Biological process	Species	Refs.
<b>Gibberellin (GA)</b>	miRNAs: 79 down, 58 up (grape)	GA biosynthesis	miR396	GRF6	Plant growth	rice	[23,28-30,33,34,41]
		GA signaling	miR159	GAMYBL2	Development	tomato, arabidopsis	
			miR159	MYB33	Flowering	arabidopsis	
			miR156	SPL	Flowering	arabidopsis	
			miR171	SCL27	Chlorophyll biosynthesis	arabidopsis	
				GRAS24	Fruit set	tomato	
<b>Brassinosteroids (BR)</b>	miRNAs: 256 (arabidopsis)	BR biosynthesis	miR397	LAC	Grain filling, panicle branching	rice	[34,36-41]
			miR444	MADS57	Root growth	rice	
			miR1848	CYP51G3	Salt response	rice	
			BR6ox-siRNA	BR6ox	Plant height, lamina bending	rice	
		BR signaling	miR159	GAMYBL2	Development	rice	
			miR396*	GRF6	Plant growth	rice	
<b>Auxin</b>	miRNAs: 30 (cotton)	Auxin biosynthesis	miR10515	SUR1	Hypocotyl growth	arabidopsis	[41,47-51,56-59,135]
			miR165/166	REV/PHB	Leaf polarity, shade-avoidance	arabidopsis	
			miR167	IAR3	Root architecture	arabidopsis	
			YUC2-siRNA	YUC2	Leaf morphology	rice	
			miR396	GRF6	Grain filling	rice	
		Auxin signaling	miR1432	ACOT	Grain filling	arabidopsis	
			miR393	TIR1/AFB	Development	arabidopsis	
			miR847	IAA28	Lateral root initiation	arabidopsis	
			tasi-ARF3/4	ARF3/4	Leaf polarity	arabidopsis	
			miR390*	TAS3	Primary root meristem	arabidopsis	
<b>Cytokinin (CK)</b>	miRNAs: 70 (balfour spruce)	CK biosynthesis	miR156	SPL	Tuber yield (potato), shoot regeneration (arabidopsis)	potato, arabidopsis	[61,63-65,67,68,71,136]
			miR159	MYB		arabidopsis	
			miR319	TCP	Development	petunia	
			Sho-siRNA	Sho		arabidopsis	
			miR165/166	REV/PHB	Root patterning and growth	arabidopsis	
			miR160	ARF10	Callus formation	arabidopsis	
		CK signaling	miR208	IPT4	Leaf senescence	tomato	
			miR171h	NSP2	Nodule initiation	medicago	
<b>Strigolactones (SL)</b>		SL signaling	miR156	SPL14	Shoot branching	rice	[74,75]
			SMXL4/5-siRNA	SMXL4/5	Anthocyanin production	arabidopsis	
<b>Salicylic acid (SA)</b>		SA signaling	miR396	GRF1	Pathogen immunity response	tomato	[82]
<b>Jasmonate (JA)</b>	sRNAs: 57 up, 24 down (arabidopsis)	JA signaling	miR319	TCP	Pathogen immunity response	arabidopsis, tomato, rice	[87-89,97,137]
	miRNAs: 189 up, 182 down (wheat)		miR156	SPL9	Insect resistance	arabidopsis	
<b>Abscisic acid (ABA)</b>	miRNAs: 14 up, 16 down (poplar)	ABA signaling	miR399f	ABF3	Drought tolerance	arabidopsis	[104-107,110,111,114,123,124,138]
	miRNAs: 107 up, 28 down (rice)		miR165/166	ABI4	Drought tolerance	arabidopsis	
	miRNAs: 26 (strawberry)		miR159	MYB33/101	Seed germination	arabidopsis	

	miRNAs: 63 up, 73 down (tomato)		miR168*	AGO1	Drought tolerance		
	miRNAs: 4 up, 29 down (knotweed)	Unknown	miR842/846	AT5G28520	Unknown		
<b>Ethylene (Et)</b>	sRNAs: 21 (tomato)	Et signaling	miR319	MYB33	Root growth	arabidopsis	[99-102, 115, 116, 120-122]
	miRNAs: 93 up, 69 down (grape)			PCF5	Salt tolerance	switchgrass	
	miRNAs: 12 up, 10 down (banana)		miR164	NAC2	Leaf senescence	arabidopsis	
	miRNAs: 8 (medicago)		miR1917	CTR4	Fruit ripen	tomato	
			miR157	SPL10	Callus proliferation	cotton	

<sup>a</sup> Abbreviations: GROWTH-REGULATING FACTOR (GRF), SCARECROW-LIKE27 (SCL27), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), SUPPRESSOR OF MAX2 1-LIKE (SMXL), PHABULOSA/REVOLUTA (PHB/REV), LACCASE (LAC), BRASSINOSTEROID UPREGULATED1, BRASSINOSTEROID-6-OXIDASE (BR6ox), AUXIN RESPONSE FACTOR (ARF), YUCCA (YUC), SUPERROOT1 (SUR1), TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB), INDOLE-3-ACETIC ACID INDUCIBLE (IAA), SHOOTMERISTEMLESS (STM), NODULATION SIGNALING PATHWAY2 (NSP2), ABRE BINDING FACTOR (ABF), ARGONAUTE1 (AGO1), PALLIATIVE CARE FORMULARY (PCF), ABA INSENSITIVE4 (ABI4), CONSTITUTIVE TRIPLE RESPONSE (CTR). \* indicates a direct regulatory connection between the hormone-responsive transcription factors and the *MIR* gene.

**Box 1. Current model for sRNA (miRNAs, siRNAs, and tRFs) biogenesis and function in plants**

In plants, miRNAs originate from *MIR* genes, which are transcribed by RNA polymerase II (Pol II) into primary miRNAs (pri-miRNAs) containing a hairpin-like structure (Figure 4A). Associated with miRNA-processing-complexes, including DICER LIKE1 (DCL1), HYPONASTIC LEAVES1 (HYL1), and SERRATE (SE), the pri-miRNAs are processed into mature miRNA duplexes [21], which are then loaded into ARGONAUTE1 (AGO1) proteins in the nucleus to form AGO:miRNA complexes with the help of HEAT SHOCK PROTEIN (HSP70/HSP90) chaperones. Via the CRM1/EXPORTIN1 (EXPO1) pathway, the AGO:miRNA complex is exported to the cytosol, where it forms part of the RNA-INDUCED SILENCING COMPLEX (RISC) [139].

Unlike miRNAs, siRNAs arise from the splicing of long double-stranded RNAs (dsRNAs) by DCL2-4 proteins into 21-24nt siRNAs, which later assemble into the RISC with AGO1 in the cytosol. These dsRNAs are produced by RNA-DEPENDENT RNA POLYMERASEs (RDRs) and SUPPRESSOR OF GENE SILENCING3 (SGS3) from aberrant transcripts or exogenous sources, including viral genomes or synthetic constructs, or by RNA POLYMERASE IV (Pol IV) and RDR2 based on single-stranded RNAs (ssRNAs) [13].

Guided by 21-22nt miRNAs or 21-24nt siRNAs, the RISC binds to complementary mRNAs and cleaves them into 5' and 3' end fragments, which are degraded by exonucleases [13]. Alternatively, some fragments are subjected to the RDR6-mediated secondary, phased siRNA (phasiRNA) biogenesis pathway [21]. For example, tasiRNAs, one type of phasiRNAs in plants, are derived from the miRNA-cleaved fragments of *TAS* transcripts [13]. Besides mRNA cleavage, miRNAs can also block ribosome-mediated translation initiation and elongation through binding to the UTR or ORF of target genes in the endoplasmic reticulum [21]. Similarly, in addition to target degradation, some 21-22nt siRNAs and most 24nt siRNAs, like hc-siRNAs, recruit methyltransferase DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) to transposable elements (TEs), transgenes or viral DNA for RdDM and transcriptional gene silencing (TGS) with the help of RNA POLYMERASE V (Pol V) [140].

Similar in size as miRNAs and siRNAs, tRFs constitute a novel class of sRNAs cleaved from mature tRNAs or as byproducts of pre-tRNA processing. Based on the cleavage



location, tRFs are categorized as tRF-5a and tRF-3a [131]. Currently, the biogenesis of tRFs in plants is unclear. Recent research showed that the level of tRFs is strongly reduced in *rns1* mutants, which are RNase T2 knockouts, but not *dcl1* mutants, implying that RNases T2 are mainly required for tRF synthesis [141,142]. Although the function of tRFs is not widely studied yet, the interaction between tRFs and AGOs suggests that they may trigger RNA silencing similarly to miRNAs and siRNAs [143].

### **Box 2. siRNAs in response to pathogen attack**

An important component in antiviral immunity is RNA silencing: the single/double-stranded viral RNAs are processed by the plants into 21-24nt virus-derived siRNAs (vsiRNAs) and also activate endogenous virus-activated siRNAs (vasiRNAs) that are synthesized via the RDR6-mediated secondary siRNA biogenesis pathway (Box 1, Figure 2) [118,144] [19]. Subsequently, vsiRNAs are incorporated in AGOs to target complementary viral RNA for degradation and vasiRNAs trigger host genes decay [145]. In the case of DNA viruses, vsiRNAs target viral DNA for RdDM [146]. In response, the viral genome encodes RSS proteins for RNA silencing suppression, such as P1/helper-component proteinase (P1/Hc-Pro) from *Potyvirus* genus, P0 from *Turnip yellows virus*, and P6 proteins from *Cauliflower mosaic virus* (CaMV). These proteins intend to compromise RNA silencing by inhibiting key components of the miRNA biogenesis pathway or attenuating AGO1/4 binding ability [147,148].

## GLOSSARY

**AGO:** ARGONAUTE family contains ten members (AGO1-10) in arabidopsis. Generally, AGO1/5/10 bind siRNAs or miRNAs and cleave mRNAs, while AGO 4/6/9 act as effectors for siRNAs-directed DNA methylation and thus induce transcriptional gene silencing.

**IPT:** adenosine phosphate-isopentenyltransferase, which catalyzes the rate-limiting step in cytokinin biosynthesis.

**PIF4:** basic helix–loop–helix transcription factor that negatively regulates photomorphogenesis and is degraded by red-light activated phyB-Pfr via physical interaction.

**PRRs:** PATTERN RECOGNITION RECEPTORS are receptors recognizing pathogen-associated molecular patterns (PAMPs) from pathogens or damage-associated molecular patterns (DAMPs) from plants, rapidly triggering immunity response.

**PTGS:** POST-TRANSCRIPTIONAL GENE SILENCING; carried out by siRNAs or miRNAs, which incorporate in AGO1 that further cleaves complementary mRNAs for RNA decay.

**PHB/REV:** PHABULOSA/REVOLUTA, two TFs belonging to the high auxin levels activating class III homeodomain leucine zipper (HD-ZIP III) transcription factors. They are directly targeted by miR165/166 and participate in modulation of tissue patterning.

**RDR:** RNA-DEPENDENT RNA POLYMERASES; the RDR family in arabidopsis consists of six proteins. Among them, RDR1/2/6 are involved in the synthesis of dsRNA molecules which are later processed to siRNAs, like vsiRNAs, hcsiRNAs, and nat-siRNAs.

**RdDM:** RNA-directed DNA methylation; an epigenetic process which is executed by 21-24nt siRNAs. These siRNAs loaded in AGO4-, AGO6-, or AGO9-RISC target to Pol V-dependent RNAs and then recruit DNA methyltransferases to methylate target genomic loci, resulting in transcriptional gene silencing.

**RNA polymerase IV and V:** both are evolved from RNA polymerase II and possess 12 subunits, which are involved in RdDM; RNA polymerase IV aids RDR2 to generate 24nt siRNAs, while RNA polymerase V transcribes genomic loci and recruits complementary siRNAs:AGO complex for cytosine methylation.

**RISC:** RNA-INDUCED SILENCING COMPLEX; a multiprotein complex comprising core unit AGO protein and other unclear members; the complex integrates with siRNAs or miRNAs to target mRNAs and then activates RNase for cleavage.

**SCL:** scarecrow-like protein, containing a conserved GRAS domain at the C-terminus. Gibberellin signaling repressors RGA and GAI belong to this family, and three SCL mRNAs (SCL6-II, SCL6-III, and SCL6-IV) are targets of miR171.

**SGS3:** SUPPRESSOR OF GENE SILENCING3; required for PTGS and belonging to an unknown protein family, which stabilizes cleavage fragments of the primary ta-siRNA transcripts and associate with RDR for the production of siRNAs.

**sRNAs:** a type of ~18 to 25 nucleotides (nt) in length non-coding RNA which appears ubiquitously in eukaryotes. They silence gene expression at transcriptional level by DNA methylation or at post-transcriptional level by cleaving mRNA transcript or by mediating translation inhibition.